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Microfungi from Portugal: *Minimelanolocus manifestus* sp. nov. and *Vermiculariopsiella pediculata* comb. nov.

Margarita Hernández-Restrepo[1*], Rafael F. Castañeda-Ruiz[2], Josepa Gené[1*], Josep Guarro[1], David W. Minter[3] & Marc Stadler[4]

[1]*Unitat de Micologia, Facultat de Medicina Ciències de la Salut, Universitat Rovira i Virgili, 43201 Reus, Tarragona, Spain*

[2]*Instituto de Investigaciones Fundamentales en Agricultura Tropical 'Alejandro de Humboldt' (INIFAT), Académico Titular de la Academia de Ciencias de Cuba, Calle 1 Esq. 2, Santiago de Las Vegas, C. Habana, Cuba, C.P.*

[3]*CABI, Bakeham Lane, Egham, Surrey, TW20 9TY, United Kingdom*

[4]*Department of Microbial Drugs, Helmholtz-Center for infection Research, Inhoffenstrasse 8,38124 Braunschweig, Germany*

*Correspondence to: *josepa.gene@urv.cat*

Abstract —Two microfungi from a forest in Portugal are described and illustrated. The new species *Minimelanolocus manifestus* is distinguished by polyblastic, integrated, sympodial conidiogenous cells, and solitary, cymbiform, (2–)3-septate, smooth conidia with pale brown middle cells and subhyaline end cells. A new combination *Vermiculariopsiella pediculata* is proposed for *Gyrothrix pediculata*.

Key words — anamorphic fungi, systematics, leaf litter

During the Iberian mycobiota study's twenty-fifth mycological foray, held in "Lagoas de Bertiandos" (Ponte de Lima, Minho province) Portugal, two interesting and unusual anamorphic fungi were collected. Individual samples of plant material were placed in paper and plastic bags, taken to the laboratory, and treated according to Castañeda-Ruiz (2005). Mounts were prepared in polyvinyl alcohol-glycerol (8 g in 100 ml of water, plus 5 ml of glycerol) and measurements made at a magnification of ×1000. Photomicrographs were obtained with a Zeiss AXIO imager M1 microscope (Göttingen, Germany). The fungus was isolated into pure culture by transferring single conidia observed under a stereo microscope onto potato-carrot agar (PCA) in Petri dishes, then incubated at 25°C under daylight, 12 h alternating cycles of light/dark. Colour notations in parentheses are from Kornerup & Wanscher (1978). The species are described below.

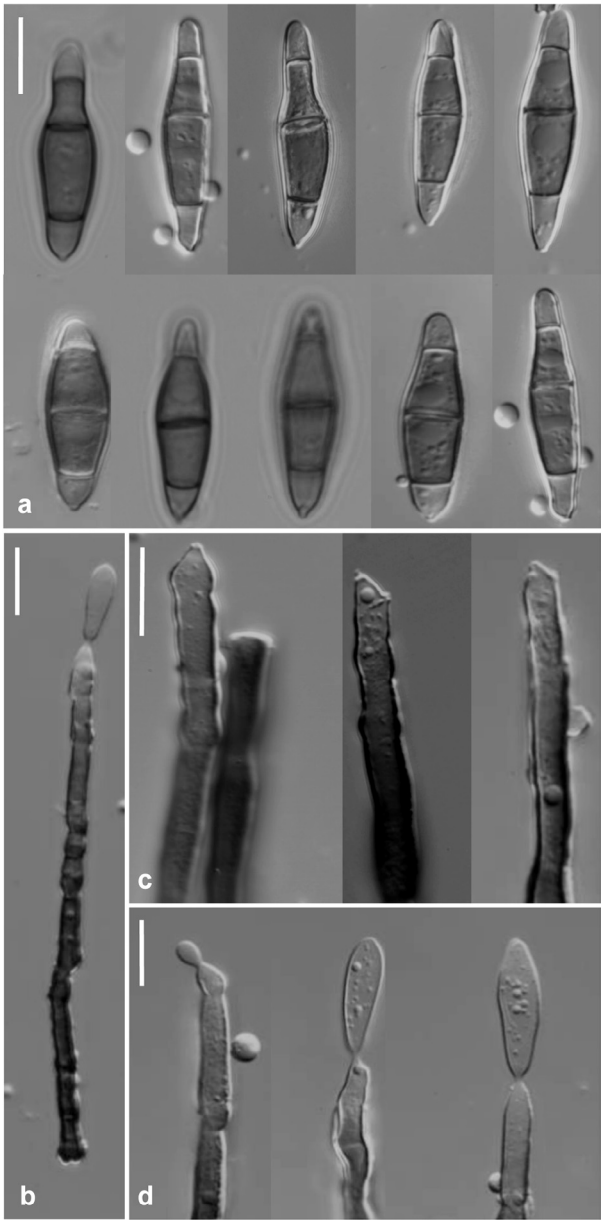

Fig 1. *Minimelanolocus manifestus* (ex holotype HAL 2450 F). a. Conidia. b. Conidiophore, conidiogenous cell and conidium. c. Conidiogenous cells. d. Conidiogenous cells and conidia. Scale bars = 10 μm.

Taxonomy

Minimelanolocus manifestus Hern.-Rest., R.F. Castañeda, Gené & Guarro, **anam. sp. nov.** Figs 1, 2

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Differs from *Minimelanolocus* spp. by conidia cymbiform to subfusiform, (2–)3-septate, with central cells pale brown and end cells subhyaline.

Type: Portugal, Minho province, "Lagoas de Bertiandos", 41°46′N 8°38′W, FMR 12182, on rotten leaf of unidentified plant, 9 November 2011, R.F. Castañeda, M. Hernández-Restrepo, J. Gené & J. Mariné-Gené (**Holotype**: HAL 2450 F).

Etymology: Latin, *manifestus*, meaning evident, clear manifest.

Colonies on the natural substratum, effuse, hairy, brown to dark brown. Mycelium superficial and immersed. Hyphae septate, branched, 2–4 µm diam., smooth, pale brown to brown. Conidiophores macronematous,

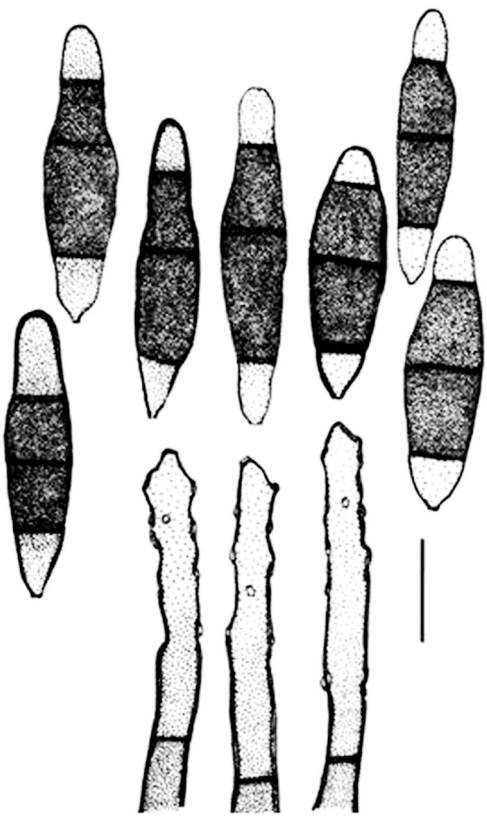

Fig 2. *Minimelanolocus manifestus* (ex holotype HAL 2450 F). Conidiogenous cells and conidia. Scale bar = 10 µm.

mononematous, erect, straight, sometimes slightly sinuate towards the apex, simple, 4–8-septate, smooth, 130–200 × 4–5 µm, brown at the base, elsewhere pale brown. Conidiogenous cells polyblastic, terminal, pale brown, 20–70 × 4–5 µm, integrated, indeterminate, with several sympodial proliferations bearing inconspicuous scars. Conidial secession schizolytic. Conidia solitary, smooth, dry, acropleurogenous, cymbiform to subfusiform, fimbrillate at the base, obtuse at the apex, (2–)3-septate, (21–)25–29 × 5–6 µm, with central cells pale brown and end cells subhyaline. Teleomorph unknown.

Note: Castañeda et al. (2001) established the genus *Minimelanolocus* with *M. navicularis* (R.F. Castañeda) R.F. Castañeda as type species to accommodate one new species and eleven combinations derived from *Pseudospiropes* M.B. Ellis, *Helminthosporium* Link, and *Belemnospora* P.M. Kirk. Subsequently, the following nine additional species were described: *M. camelliae* H.B. Fu & X.G. Zhang, *M. chimonanthi* Y.D. Zhang & X.G. Zhang, *M. endospermi* Jian Ma & X.G. Zhang, *M. linderae* Jian Ma & X.G. Zhang, *M. machili* K. Zhang & X.G. Zhang, *M. magnoliae* K. Zhang & X.G. Zhang, *M. mori* Jian Ma & X.G. Zhang, *M. olivaceus* R.F. Castañeda & Guarro, and *M. pterocarpi* Jian Ma & X.G. Zhang (Castañeda et al. 2003, Ma et al. 2008, 2011, Zhang et al. 2009, 2010). Among the known species, *M. manifestus* most closely resembles *M. linderae* and *M. navicularis*, but *M. linderae* has ellipsoidal or cylindrical conidia, 26–35 × 7–9 µm, which are mostly 4-septate and rarely 3-septate, while *M. navicularis* possesses navicular to sub-rhombic conidia, 20–25 × 6–8 µm, which are (2–)3(–5)-septate, with brown to dark brown central cells and subhyaline end cells. All three species can be easily differentiated.

Vermiculariopsiella pediculata (J.L. Cunn.) Hern.-Rest., R.F. Castañeda, Gené & Guarro, **comb. nov.** Figs 3–5

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≡ *Gyrothrix pediculata* J.L. Cunn., Mycologia 66(1): 123 (1974).

Anamorphic fungus. Colonies on the natural substratum effuse, blister-shaped to sporodochial, hairy, whitish, amphigenous. Mycelium superficial and immersed. Hyphae septate, frequently anastomosing, 1–3 µm diam. Setae erect, cylindrical, 110–220 × 4–6 µm, but wider, 5–9 µm diam at the base, with alternate, irregular to imperfect dichotomous branches, the branches being attenuate, coiled to whip-like, curved, septate, smooth, brown below, and pale brown at the apex. Conidiophores comprised solely of conidiogenous cells. Conidiogenous cells monophialidic, discrete, ampulliform to subulate, sometimes very close packed forming a cespitose layer, pale brown at the base, subhyaline at the apex, 14–15 × 3–4 µm, with a distinct, narrow, refractive collarette. Conidia asymmetrical, sub-fusiform to navicular or sub-oblong, attenuate, somewhat apiculate, curved at the apex, tumid, somewhat irregular,

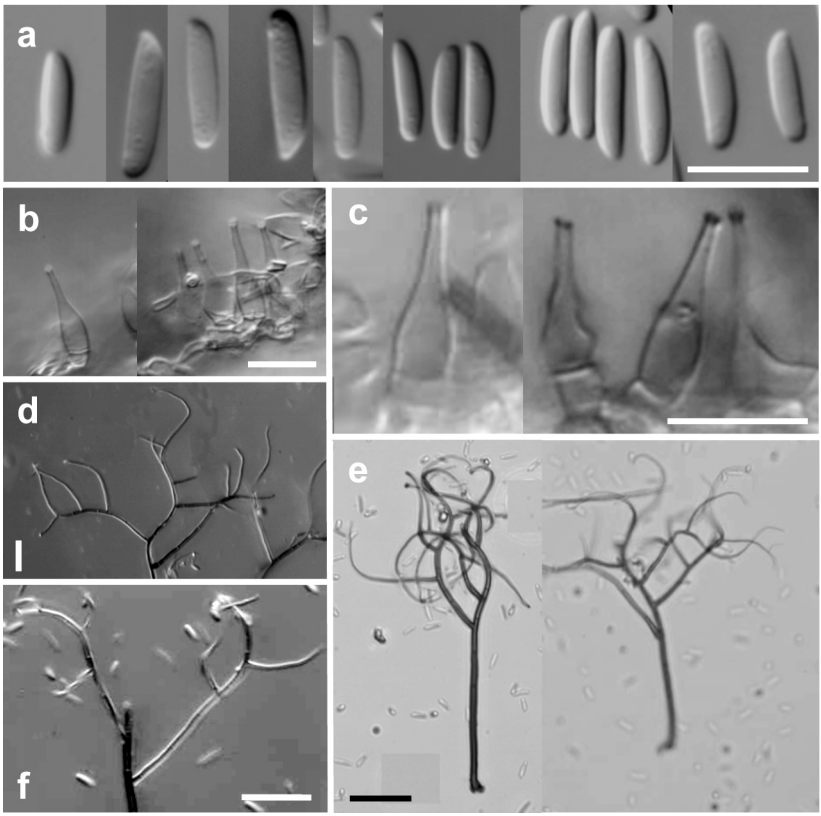

Fig 3. *Vermiculariopsiella pediculata* (ex nature HAL 2447 F). a. Conidia. b, c. Conidiogenous cells. d-f. Setae. Scale bars = 10 µm.

eccentrically and inconspicuously fimbrillate at the base, unicellular, hyaline, smooth, 5–9 × 2 µm, produced seriately and obliquely from the conidiogenous loci, forming a white cirrhus or pseudochains in snow-like masses.

Specimen examined: **PORTUGAL**, **Minho province**, "Lagoas de Bertiandos", 41°46′N 8°38′W, FMR 12187, on rotten leaf of unidentified plant, 9 November 2011, R.F. Castañeda, M. Hernández-Restrepo, J. Gené & J. Mariné-Gené (HAL 2447 F).

Colonies on PCA, attaining 21–30 mm after 7 days at 25°C, floccose, raw umber (5F8) with yellowish white (4A2) and numerous white pustules somewhat pulvinate formed by conidial cirrhus. Setae similar to those on the natural substratum, 100–149 × 4–6 µm. Hyphae septate, subhyaline to very pale brown, 1–2 µm diam, smooth. Conidiophores comprised solely of conidiogenous cells. Conidiogenous cells monophialidic, subulate to ampulliform, slightly sinuate or curved, very pale brown at the base, hyaline

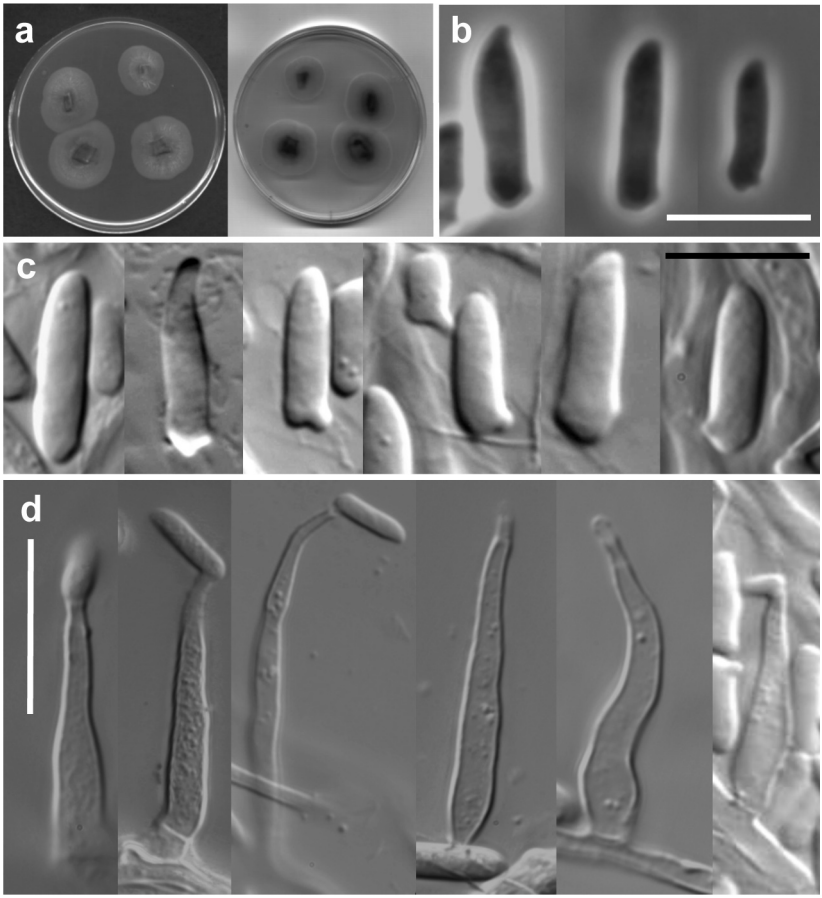

Fig 4. *Vermiculariopsiella pediculata* (ex culture HAL 2451 F). a. Colonies on PCA (obverse and reverse, respectively) at 25ºC after 7 days. b, c. Conidia. d. Conidiogenous cells. Scale bars = 10 µm.

towards the apex, 19–28 × 3–4 µm. Conidia asymmetrical obclavate to long obovoid, oblong or irregular, obtuse, slightly curved or subuncinate at the apex, inflated, tumid, eccentrically fimbrillate at the base, hyaline, 19–28 × 3–4 µm, smooth-walled, produced axially at the conidiogenous cell apex in a long white cirrhus or pseudocatenate.

Cultures deposited: (ex HAL 2447 F) HAL 2451 F, CBS 132484, FMR 12187.

Note: Nakagiri & Ito (1991) illustrated the conidiogenous cells and conidium ontogeny from *Gyrothrix circinata* (Berk. & M.A. Curtis) S. Hughes and regarded conidial production as "phialides type"; the authors interpreted the whorled arrangement of conidia at the conidiogenous cell apex as resulting

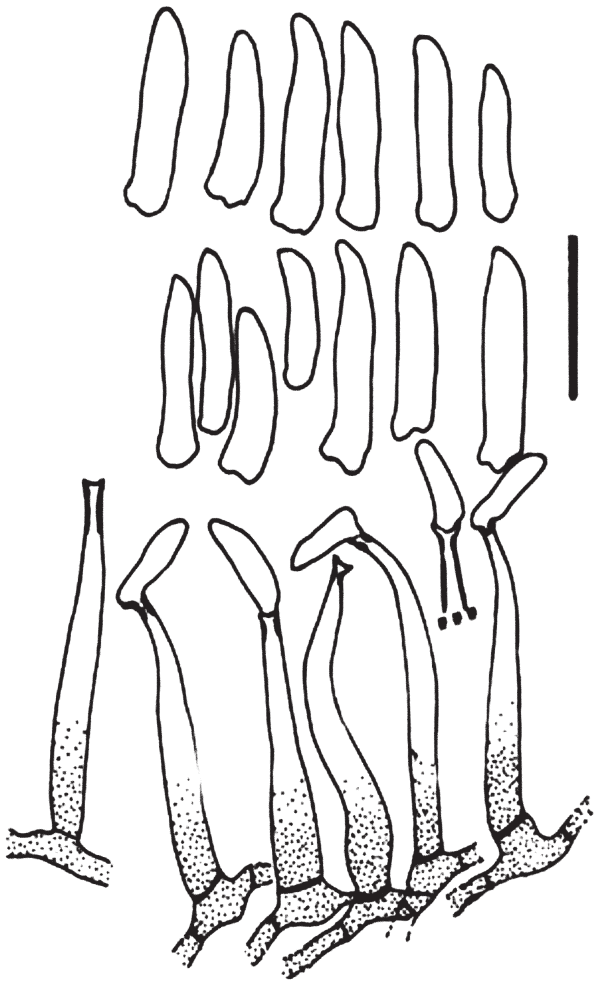

Fig 5. *Vermiculariopsiella pediculata* (ex culture on PCA, HAL 2451 F).
Conidia and conidiogenous cells. Scale bar = 10 µm.

from mucilaginous material produced by and accumulated at the tip of each conidiogenous cell. Crous et al. (1996), working with *Gyrothrix verticiclada* (Goid.) S. Hughes & Piroz., illustrated inconspicuous annellations at the conidiogenous cell apices produced after enteroblastic percurrent proliferations. The aggregated conidia forming a whitish, more or less uniform layer at the base of each seta is clearly the result of a conidial ontogeny with successive enteroblastic percurrent or sympodial proliferations occurring near the apex

of each conidiogenous cell, but the number of conidia produced by this way is apparently limited and can be differentiated from the conidia produced from a "phialide" as described by Kirk et al. (2008).

Wu et al. (1997) suggested that conidiogenous cells in *Ceratocladium*, *Circinotrichum,* and *Gyrothrix* are not typical "phialides". Cunningham (1974) described and illustrated the conidiogenous events on *Gyrothrix pediculata* from cultures on corn meal agar. This pattern of ontogeny was classified as conidial development type 18 (conidium ontogeny holoblastic, delimitation by 1 septum, schizolytic secession, maturation by diffuse wall-building, percurrent and sympodial enteroblastic conidiogenous cell proliferation below the previous locus and delimiting septum, the second and subsequent conidia formed from proliferations, but with no observed reduction in length of the conidiogenous cells). Setose conidiomata with branched setae and similar conidial ontogeny can be found in *Vermiculariopsiella* Bender [*V. cornuta* (V. Rao & de Hoog) Nawawi, Kuthub. & B. Sutton, *V. cubensis* (R.F. Castañeda) Nawawi, Kuthub. & B. Sutton*, V. microsperma* (Höhn.) R.F. Castañeda & W.B. Kendr.*,* and *V. ramosa* (B. Sutton) Nawawi, Kuthub. & B. Sutton]. As "phialides" were described for the conidiogenous cells in *Vermiculariopsiella* by Morgan-Jones et al. (1972), Nawawi et al. (1990) and Keshavaprasad et al. (2003), the transfer of *G. pediculata* to *Vermiculariopsiella* is clearly supported and agrees with the generic concept.

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